PB82-155292

Viral Diseases of Invertebrates Other Than Insects

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

Dec 81

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TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
ERLGB 0060		
1. REPORT NO. EPA-600/D-80-055	2.	3. RE PB82-155292
4. TITLE AND SUBTITLE VIRAL DISEASES OF INVERTEBRATES OTHER THAN INSECTS		5. REPORT DATE December 1981 6. PERFORMING ORGANIZATION CODE 026081
7. AUTHOR(S) John A. Couch		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Enviornmental Protection Agency Environmental Research Laboratory Office of Research and Development Gulf Breeze, FL 32561		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

In: Pathogenesis of Invertebrate Microbial Diseases, Davidson, ed., Allanheld, Osmun, and Co., publishers, Totowa, N. J., p. 127-160

16. ABSTRACT

Thirteen examples of virus or viruslike related pathoses in non-insect invertebrates are described. From consideration of these examples, it becomes obvious that detailed descriptions of pathogenesis of virus diseases in non-insect invertebrates has not kept pace with the frequency of new reports of viruses from these invertebrates. When possible, it is of utmost importance for authors reporting viral entities from invertebrates to include as much information as can be collected on the disease syndrome, cellular pathogenesis, physiological and behavioral effects and tissue distribution of the virus. In some cases, information has been so scant that readers are not even certain as to the prevalence of the virus in reported hosts.

Basic information on the nature of non-insect invertebrate virus diseases is needed for several reasons. Insights into mechanisms of pathogenesis and pathogenic effects in invertebrate viruses may give better understanding of vertebrate virus diseases. Further, a major opportunity exists with invertebrate virus disease to investigate the fundamental aspects of cellular immunity.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
	Pathogenesis Viral Diseases Invertebrates Viral Diseases of Crustacea Shrimp Diseases Non-insect Microbial Diseases	Biological Pesticides Biorationals

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	Unclassified	33
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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CHAPTER 5

VIRAL DISEASES OF INVERTEBRATES OTHER THAN INSECTS

by John A. Couch*

INTRODUCTION

Considerable recent interest has focused on the discovery of viruses that cause diseases in non-insect invertebrates. Since Vago (1966) reported the first virus disease of a marine crustacean, viruses and/or viral diseases have been reported for shrimps, crabs, daphnids, entoniscid isopods, oysters of several genera, octopods and squids, annelids, trematodes, nematodes, hydra, and a sponge. Most of the reports contained only brief descriptions of the virus with little information on pathogenesis in the hosts. A few reports have been more extensive and contain detailed descriptions of virus-related pathogenesis and virus-host cell relationships.

This chapter will describe some aspects of the pathogenesis of better known virus diseases in several exemplary non-insect invertebrate hosts. Also included will be brief descriptions of some lesser known viruses in other invertebrates to demonstrate the range of relationships among viral groups and non-insect invertebrates. Available information on the histopathogenesis, cellular pathogenesis, physiological, and behavioral responses to select host-virus interactions will be discussed under the headings of virus/host types (Table 5-1).

*United States Environmental Protection Agency, Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561

PATHOGENESIS OF INVERTEBRATE MICROBIAL DISEASES. Davidson, ed. Allanheld, Osmun (Totowa, N.J.)

TABLE 5-1

A PARTIAL LIST OF VIRUS/HOST PATHOLOGICAL RELATIONSHIPS IN NON-INSECT INVERTEBRATES
(STARRED VIRUSES * ARE DISCUSSED IN GREATER DETAIL IN TEXT)

Virus[1]	Host	Tissue/cell type	Reference
*Baculovirus	Penaeid shrimps (Penaeus spp.)	hepatopancreas epithelial cells; midgut	Couch, 1974b
Baculovirus	Penaeid shrimp (Penaeus monodon)	hepatopancreas epithelial cells	Lightner & Redman, 1981 Lightner et al.,1981
*Baculovirus	Blue crab (Callinectes sapidus)	hepatopancreas	Johnson, 1976b
*Baculovirus	Crabs (Carcinus mediterraneus) (Carcinus maenas)	epithelial cells, hepatopancreas	Bazin et al., 1974
*Spherical virus	Carcinus maenas	hemocytes	Bang, 1971
Paramyovirus or Bunyavirus	Swimming Crab (Macropipus depurator)	hepatopancreas cells; hemocytes	Bonami & Vago, 1971
Paralysis virus (Reovirus)	Swimming Crab (M. depurator)	hemocytes	Bonami, J. R. 1973
*Picornavirus	Isopod (Portunion conformis) Shore crab (Hemigrapsus oregonensis)	hepatopancreas	Kuris et al, 1979
*Herpes like virus	Blue crab (C. sapidus)	hemocytes; gill epithelium	Johnson, 1976b
*Rhabdolike virus	Blue crab (C. sapidus)	cells of mandibular gland; hemocytes	Yudin & Clar, 1979
Paramyxovirus (enveloped helical virus)	Blue crab (C. sapidus)	hemocytes; hemopoietic tissue	Johnson & Farley, 1980
*Reolike virus	Blue crab (C. sapidus)	hemocytes; hemopoietic tissue	Johnson & Bodammer, 1975

Iridovirus	Daphnid (Simocephalus expinosus)	adipose and glandular tissue; nerve	Federici & Hazard, 1975
Cytoplasmic Polyhedrosis virus	Daphnid (S. expinosus)	adipose and glandular tissue; nerve	
*Herpes-like virus	American Oyster (Crassostrea virginica)	midgut epithelial cells; hemocytes (?)	Farley et al., 1972
*Hexagonal virus	Pacific oyster (larvae) (Crassostrea gigas)	velar epithelial cells	Elston, 1979, 1980
*Phage	Rickettsia parasitic in the marine bivalve, Tellinia tenuis	Rickettsia, host of phage in cytoplasm of digestive gland cells of bivalve	Buchanan, 1978
Reovirus	Squid (Sepia officinalis)	stomach epithelial cells	Devauchelle & Vago, 1971
*Hexagonal virus like particles	Octopus vulgaris	ventral mantle; arms; in tumors	Rungger et al., 1977
*Spherical virus like particles	Nematode (Trichosomoides crassicauda)	somatic cells (hypodermal cells)	
*Adenovirus	Hydra vulgaris	epithelial cells	Bonnefoy et al., 1972
Adenovirus	Sponge (Verongia cavernicola)	giant cells (possibly degenerating oocytes)	Vacelet and Gullissian, 1978

1 Group or taxonomic designations for viruses are those used or suggested by original authors and may be subject to change as more is learned of these viruses.

BACULOVIRUS INFECTIONS IN PENAEID SHRIMPS

Until 1973 Baculovirus infections (including nuclear polyhedrosis viruses) were unknown in hosts other than insects and mites (see Chapter 1). During 1973 and 1974 two Baculovirus forms were reported, one in penaeid shrimps (Couch and Niumo, 1973; Couch 1974a) and one in crabs (Bazin et al., 1974). Work on the shrimp virus distribution (Couch et al., 1975; Couch, 1978), ecology (Couch et al., 1975), ultrastructure (Couch, 1974 a, b, 1978) cytopathic effects (Couch, 1974 a, b; Couch et al., 1975; Couch, 1978), and physical and biochemical characterization (Summers, 1977) has been considerable. Therefore, this virus-host system will be described in some detail.

Histopathogenesis

Hepatopancreatic epithelial cells are the major tissue type infected in the following penaeid shrimps: pink shrimp, Penaeus duorarum; brown shrimp, Penaeus aztecus; white shrimp, Penaeus setiferus; and possibly the Pacific penaeid, Penaeus monodon. Infections occur in the nucleus, and are evident with light microscopy (Couch, 1974 a, b; Couch, et al., 1975) because of the formation of large intranuclear, tetrahedral inclusion bodies (polyhedral inclusions - PIB's) in the presence of rod-shaped virions (Figs. 5-1, 5-2). The major results of histopathologic damage in infections are destruction of segments or areas of hepatopancreatic tubule epithelium by growth of PIB's in and eruptions from individual cells (Figs. 5-3, 5-4).

Cellular and ultrastructural pathogenesis

Table 5-2 presents the nuclear and cytoplasmic virus-associated changes that occur in the chronological order of their appearance in infected shrimp cells. Infected hepatopancreatic cells may show bizarre departures from normal in both the nucleus and cytoplasm (Figs. 5-5, 5-6).

The first indication of infection at the cellular level is hypertrophy of the hepatopancreatic epithelial cell nucleus to two to five times normal diameter (Fig. 5-7). Nuclear hypertrophy is not usually accompanied by cytoplasmic increase; therefore, the nucleus is obviously enlarged. Diminution and margination of heterochromatin next occurs (Figs. 5-8). Eventually all heterochromatin disappears from the nucleus (Fig. 5-9). Concurrent with loss of heterochromatin is the appearance of fibrillar and granular stromata in the nucleoplasm (Figs. 5-5, 5-8, 5-9). Soon after this, the

virions appear and growth of the tetrahedral inclusion body begins (Fig. 5-6).

Cytoplasmic changes associated with virus replication and synthesis are extreme and unusual, even in comparison with known cytopathic effects of other viruses. The most striking cytoplasmic event in infection is the extreme proliferation of the outer nuclear membrane resulting in the formation of the membranous labyrinth within the cytoplasm (Couch, 1974a) (Figs. 5-5, 5-6, 5-8). This membrane proliferation is so distinctive that at the ultrastructural level it is pathognomic of Baculovirus infections in shrimp cells. Myelenoid body formation in the cytoplasm may result partially from the collapse of proliferated nuclear membranes into concentric masses (Fig. 5-6). Mitochondria become enlarged and distorted as nuclear replication of viruses occurs (Fig. 5-6); this probably results from loss of membrane permeability control and osmotic balance controls in the infected cell. The second most striking cytoplasmic change, after nuclear membrane proliferation, is the loss of larger organelles in advanced infections concomitant with all cytoplasmic space becoming filled with free ribosomes (Fig. 5-9). At this stage, the cell may lyse or become necrotic.

Physiological effects

Few physiological characteristics of shrimps have been investigated in relationship to Baculovirus infections. From studies to date, however, there appear to be two important functional effects.

The first is the direct, observable effect of viral infection, i.e. replication in, and destruction of vital hepatopancreatic epithelial cells. Extensive epithelial tissues are destroyed when infected cells are lysed (Figs. 5-1, 5-3). The loss of tubular embryonic cells, absorptive cells, and secretory cells in the hepatopancreas leads to the loss of the major functions of this important organ. The loss of tubular growth capacity, nutrient absorption, and secretory, digestive functions may constitute the major, direct dysfunctional results of infection. Further, infected larval shrimp may lose, at a critical period in their growth, the majority of their early, developing hepatopancreas.

The second functional effect is less directly observed and probably involves an interaction between the virus and stress moderated effects in the host. Couch and Courtney (1977) demonstrated that shrimp that were sublethally stressed with the toxic polychorinated biphenyl (PCB) Aroclor 1254 were more susceptible to infection and spread of the Baculovirus than were non-stressed shrimp. The process causing this loss of resistance to viral spread is not known, but may involve

Figure 5-1. Baculovirus polyhedral inclusion bodies (PIB's) in, and free of, epithelial cells from hepatopancreas of penaeid shrimp, Penaeus duorarum (Phase contrast).

Figure 5-2. Tetrahedral PIB's released from shrimp cells (Phase contrast).

Figure 5-3. Rupture and release of PIB's from heavily Baculovirus infected hepatopancreas epithelium of P. duorarum.

20µm
1
2
15µm
3

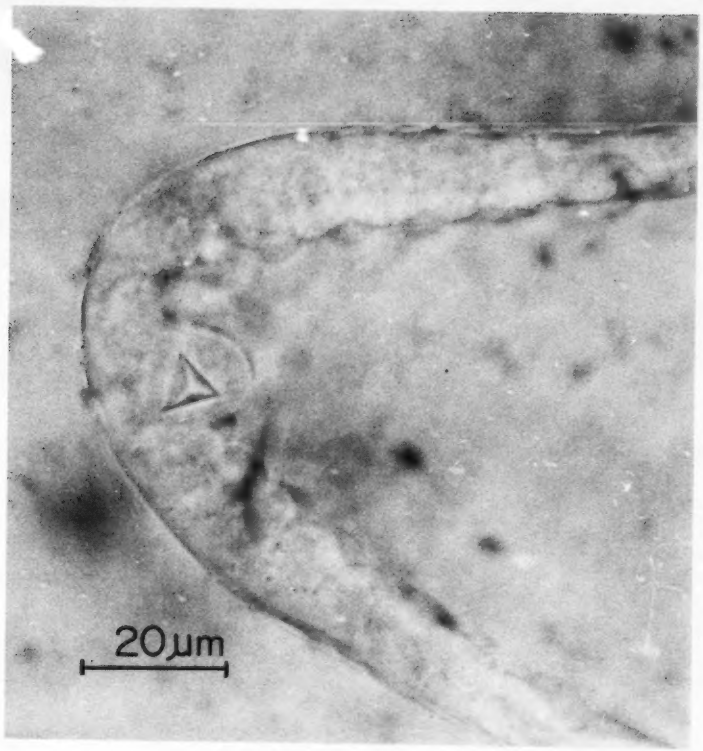

Figure 5-4. Acinus of hepatop reas from Penaeus duorarum with a single embryonic cell nucleus (epithelium) containing a large PIB. Growth of the PIB will soon rupture the cell freeing the virion-laden PIB.

TABLE 5-2

CELLULAR PATHOGENESIS IN BACULOVIRUS-INFECTED SHRIMP CELLS

Nuclear	Cytoplasmic
1. Nuclear hypertrophy	1. Cytoplasmic diminution
2. Heterochromatin diminution	2. Formation of membranous labyrinth[1]
3. Heterochromatin margination	3. Myelenoid bodies formed
4. Disappearance of heterochromatin	4. Mitochondria enlarged, distorted
5. Nucleolar loss	5. ER vesicles enlarged
6. Nucleoplasm fibrillar stroma and granular stroma	6. Cytoplasm filled with free ribosomes
7. Nuclear membrane proliferation[1]	7. Disappearance of all organelles
8. Appearance and growth of polyhedral inclusion body (PIB)	
9. Virion-associated membranes and materials present in nucleus	

[1]These nuclear and cytoplasmic events are interrelated, i.e. the membranous labyrinth of the cytoplasm is generated by the proliferation of outer nuclear membrane (Fig. 5-5) (Couch, unpub.).

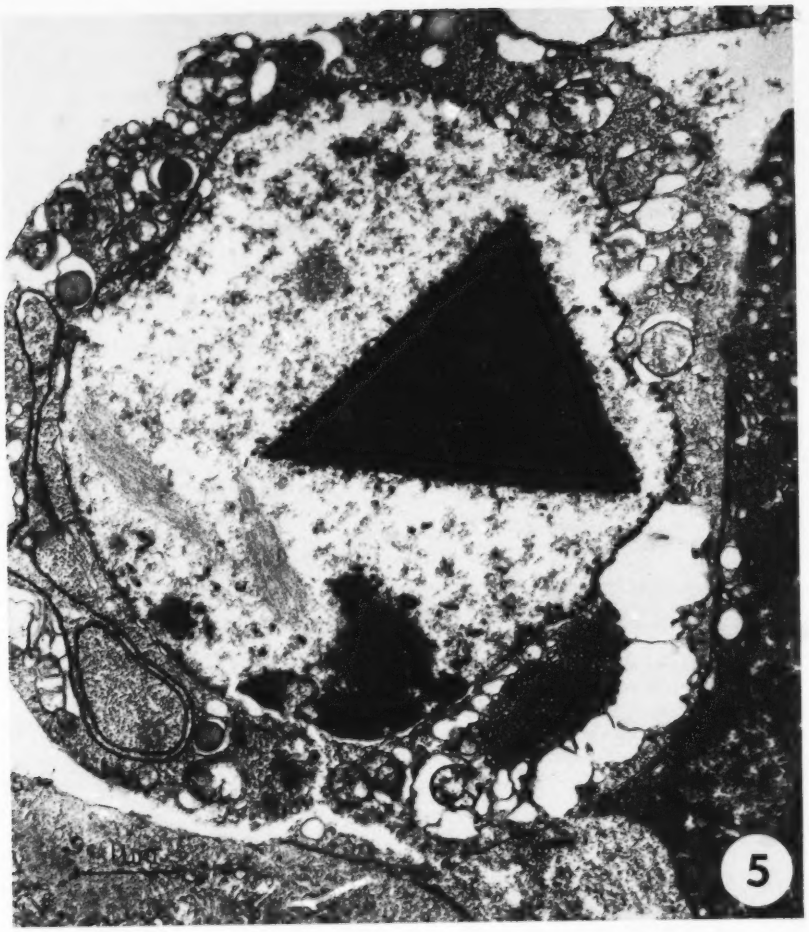

Figure 5-5. Electron micrograph of hepatopancreatic cell infected with Baculovirus penaei; note the nuclear hypertrophy, heterochromatin diminution, and growth of large PIB in nucleoplasm with fibrillar and granular stromata in nucleus; cytoplasmic changes are obvious and consist of membrane proliferation or formation of membranous labyrinth which is pathognomonic for the shrimp Baculovirus, mitochondrial swelling, and free ribosome increase. Note virions adjacent to inner layer of nuclear envelope.

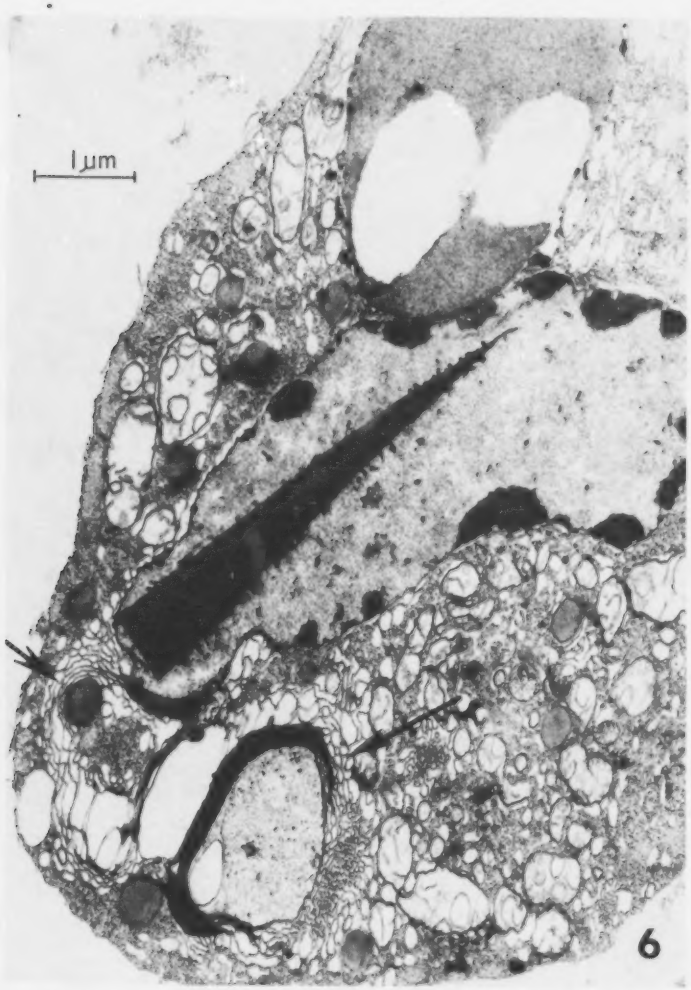

Figure 5-6. Electron micrograph of hepatopancreatic cell showing unusual place of section: note the peripheral section of PIB in nucleus; sharp edges and apices of PIB's serve to rupture nuclear and cytoplasmic membranes effecting the release of the virion-containing PIB. Cytoplasmic changes include bizarre membrane alterations and formation of myeloid bodies (arrow).

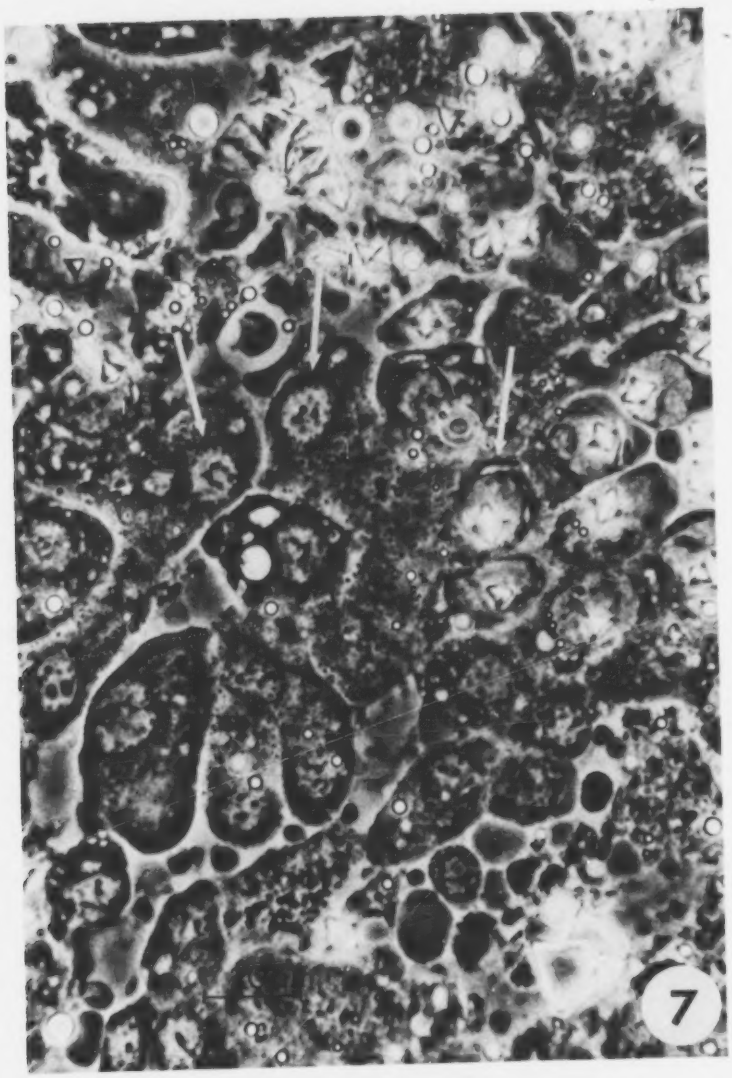

Figure 5-7. Nuclear hypertrophy of Baculovirus-infected hepatopancreas cells (arrows); note that this figure contains several stages of infected cells from non-patent to patent infections. (Phase contrast).

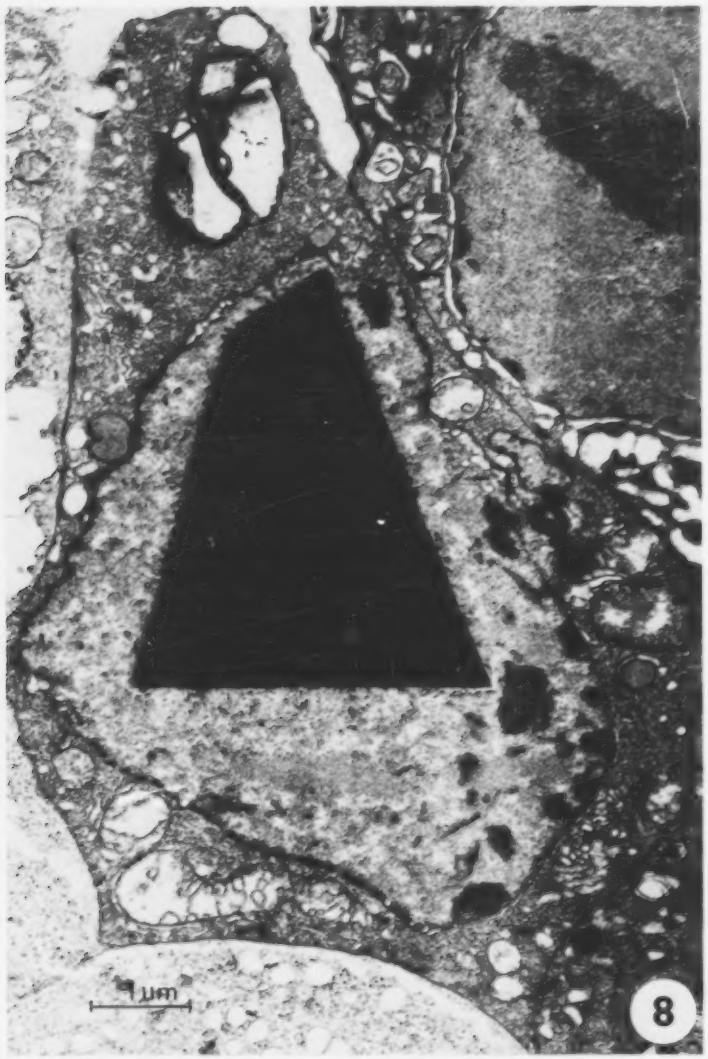

Figure 5-8. Margination and diminution of heterochromatin is apparent in this nucleus infected by Baculovirus penaei; note large, semitruncated PIB in section and virions distributed around PIB; enlarged or swollen mitochondria are apparent in cytoplasm.

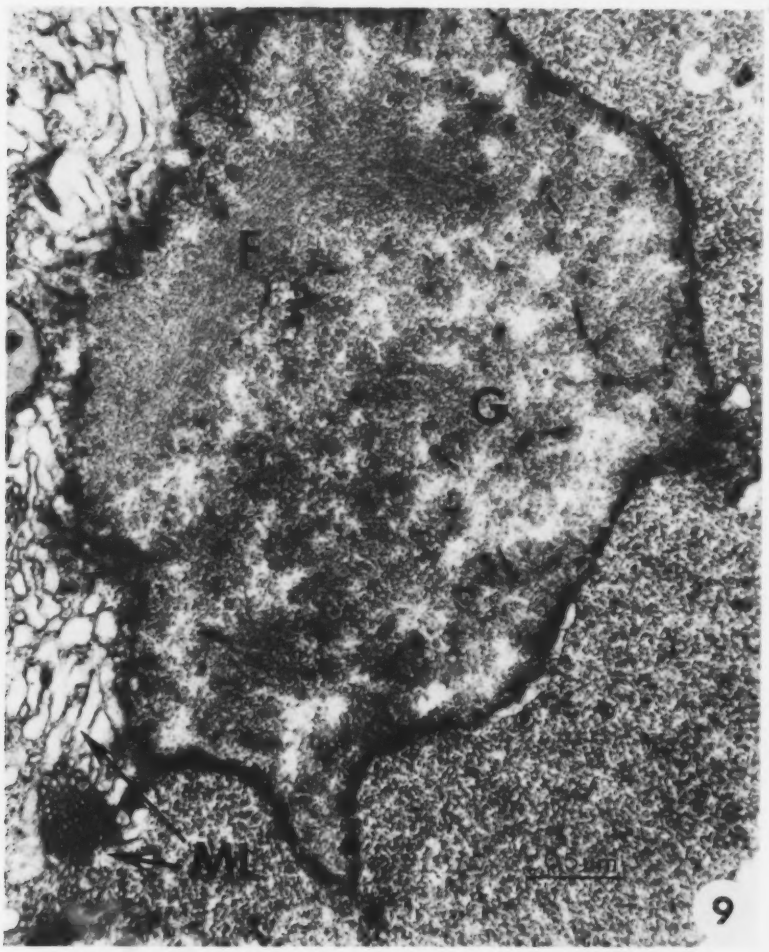

Figure 5-9. Oblique section through nucleus excluding the PIB in advanced, Baculovirus-infected cell from shrimp; note fibrillar (F) and granular (G) stromata in nucleus, with virions attached to inner nuclear envelope; cytoplasm is filled with free ribosomes and membranous labyrinth (ML:proliferated outer nuclear envelope) characteristic of this virus infection in shrimp.

chemical or toxic inhibition of innate cellular defense mechanisms, e.g. phagocytosis, interferon production, or secretory functions.

BACULOVIRUS INFECTIONS IN THE BLUE CRAB AND IN THE EUROPEAN SHORE CRAB

Johnson (1976a) reported a Baculovirus in nuclei of epithelial cells of the hepatopancreas in blue crabs (Callinectes sapidus) (Fig. 5-10). Earlier, Bazin et al. (1974) reported a Baculovirus in the nuclei of the hemocytes and connective tissue cells of the shore crab, Carcinus maenas. These two viruses appear to be more similar to one another than either is to the shrimp Baculovirus. A major difference between the shrimp Baculovirus and the crab baculoviruses is that the latter are not associated with inclusion bodies in the host cell nucleus (compare Fig. 5-5 with Fig. 5-10). There also appear to be differences as well as a few similarities in the host cell cytopathic responses between the crab and shrimp baculovirus infections. The major similarity between host cellular response to infection between the shrimp and crab baculoviruses is the chromatin diminution and margination concomitant with nuclear hypertrophy (Figs. 5-5, 5-10, and Fig. 1 in Bazin et al., 1974). Host cellular responses differ significantly, however, in lack of membrane proliferation, and other cytoplasmic changes in crab baculovirus infections. Perhaps not as much material has been examined in the crab infections compared to large numbers of infected shrimp studied, thus giving an incomplete picture of the crab cellular response.

Baculovirus infections in the above crabs have not yet been associated with fatal disease, whereas considerable evidence exists for lethal infections in shrimp, particularly larval shrimp (Couch, 1978). However, Pappalardo et al. (1979) have reported a Baculovirus in hepatopancreatic cells of the crab Carcinus meditteraneus that may cause lethal disease. Little detail of pathogenesis was given in the original report.

SPHERICAL VIRUS IN SHORE CRAB, CARCINUS MAENAS

Bang (1971) reported a transmissable infection associated with a spherical virus-like particle of varying size (55-125 nm) in the European shore crab, Carcinus maenas. It is considered here because Bang (1971) gave considerable information on pathologic changes in hemolymph, hemocyte, and tissue characteristics of the host, and on successful transmission of the agent to previously uninfected crabs in the laboratory. Hemolymph from an original infected crab was inoculated into many other crabs, producing disease similar to that in the

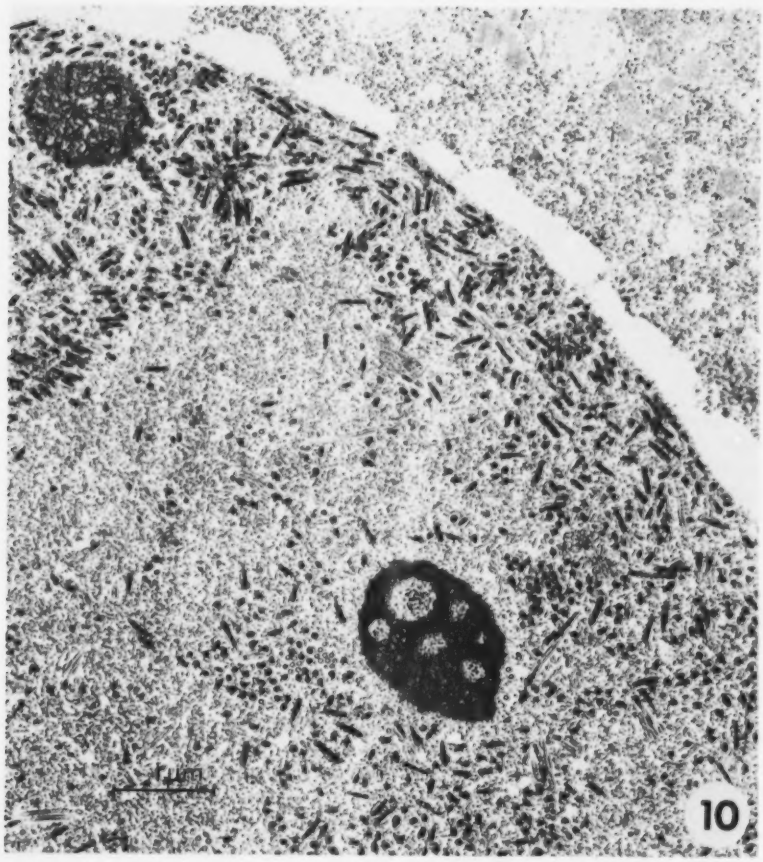

Figure 5-10. Baculovirus in nucleus of hepatopancreatic cell from blue crab (Callinectes sapidus); note lack of inclusion body - compare with Figs. 5-5, 5-6 and 5-8 of shrimp Baculovirus; note nucleolar remnants in nucleus (micrograph supplied by P. T. Johnson).

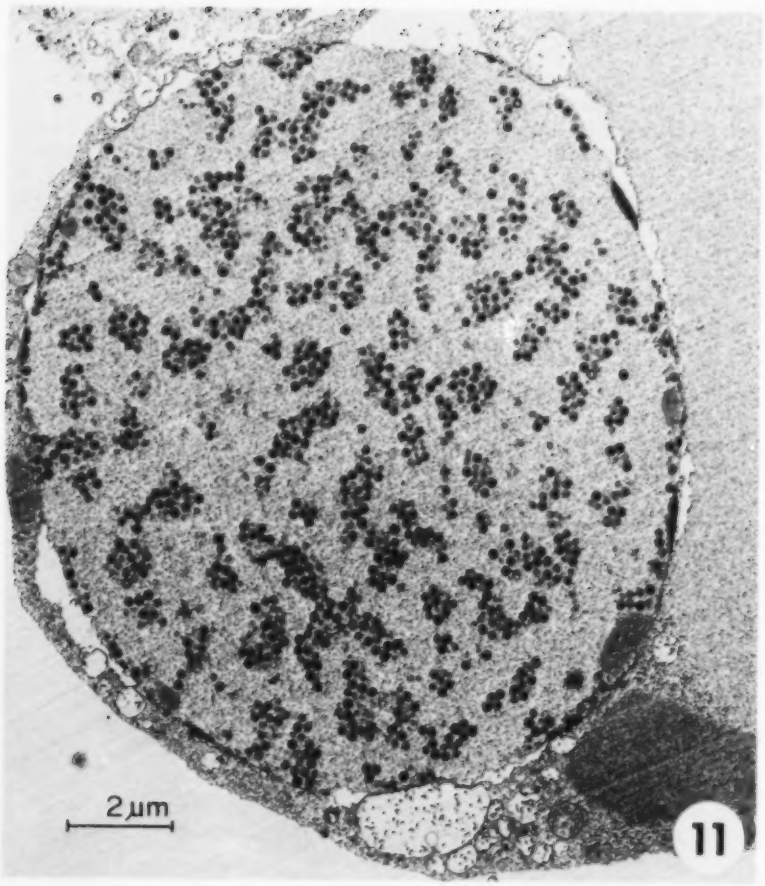

Figure 5-11. Herpes-like virus in hemocyte of the blue crab; note heterochromatin diminution, nuclear hypertrophy, and absence of nucleolus; virions occur in clusters as completely and partially formed particles; perinuclear vacuolization and cytoplasmic lysis are suggested in micrograph (Micrograph supplied by P. T. Johnson).

original crab.

General pathology

The major pathologic change in afflicted crabs was the loss of hemolymph clotting ability, which in crustacea is controlled or mediated by hemocytes or blood cells. Following inoculation, crabs that became infected showed loss of blood clotting in from 2 to 19 days. Seventy-four percent of 73 crabs inoculated with known infected blood developed a failure to form blood clots (Bang, 1971). The following differences were found between the blood cells of the normal and infected crabs: (i) decrease in blood cells numbers in affected crabs in comparison to blood cell numbers in normal crabs; (ii) morphological differences between infected crab blood cells and those of normal crabs consisting of fewer filaments, altered ectoplasmic form, fewer granules per cell and transition from a normal granular appearance to a hyaline appearance; and (iii) loss of ability to secrete fibrinogen. This latter abnormality probably provides the basis for loss of clotting capacity of the blood in infected animals.

Most evidence including some limited electron microscopy, indicates that a filterable, spherical virus is the agent responsible for the disease. However, only limited electron microscopy of this virus has been reported; therefore, little information is available on ultrastructural effects of the agent and or actual identification or affinity of the virus. With electron microscopy the virus was found in blood cells in the endoplasmic reticulum and associated with cell membranes (Bang, 1971).

HERPESLIKE VIRUS IN THE BLUE CRAB

Johnson (1976 a, b) reported herpeslike virus particles in nuclei of hemopoietic cells, hemocytes, and gill epithelial cells of the blue crab from Virginia, USA.

General pathology

Infected cells were recognized because of gross hypertrophy of nuclei visible with the light microscope. Cowdry Type-A inclusions were present in some of the nuclei. Nuclei lacking cytoplasm also were found in acellular spaces, and lysed or partially lysed cells were found. Electron microscopy revealed that enlarged, infected nuclei contained many typical herpeslike virions (Fig. 5-11). Infected nuclei were altered further in that most of the heterochromatin was gone in the hypertrophied nuclei, nucleoli were absent, and

the nucleoplasm appeared fine and granular surrounding clusters of virions (Fig. 5-11). Cytoplasmic effects included vacuolization adjunct to nuclear membranes, swelling of perinuclear space (between nuclear envelope membranes), loss of cytoplasmic organelles or their reduction in number, and general lysis of cytoplasm to a granular homogeneity (Fig. 5-11).

No information was presented on the systemic effect of the virus in the crabs. Destruction of hemotopoietic cells might lead to anemia if lysis of blood cell progenitor cells were extensive enough. No evidence was reported for such a condition. Johnson (1976a) did report finding several juvenile crabs infected from Chincoteague Bay, Virginia.

RHABDOVIRUS-LIKE PARTICLES IN THE BLUE CRAB

Yudin and Clark (1979) have described a Rhabdovirus that occurs in mandibular gland cells and hemocytes following ablation of eyestalks in the blue crab. The virus-like particles appeared within the nuclear envelope and tubular endoplasmic reticulum of gland cells during days 3, 4, and 5 following ablation.

General pathology

At the light microscopic level infected gland cells and hemocytes could be distinguished from non-infected by the presence of large accumulations of virus particles in the cytoplasm. Though individual virions were not visible, their aggregates were conspicuous. No other histopathologic signs were reported for infected crabs.

With the electron microscope, Yudin and Clark (1979) found significant cellular changes associated with the presence and development of virus particles. They found that virus proliferation was associated with three organelle systems: the nuclear envelope, the tubular endoplasmic reticulum (TER) characteristic of the mandibular gland cells, and the cell membrane. Effects on these organelles or upon the cell involved the appearance of virus-filled vacuoles and enlargement of TER prior to or as it filled with particles. In hemocytes, two effects were the replacement of normal cytoplasmic organelles and necrosis of the nucleus. Fig. 5-12 shows the ultrastructural effects of virus proliferation in hemocytes of the blue crab.

The Rhabdovirus-like particles have been reported only in blue crabs that have undergone eyestalk ablation. Yudin and Clark (1979) alluded that a stress related condition or even an endocrine imbalance in these ablated animals may have enhanced or precipitated the virus expression and proliferation.

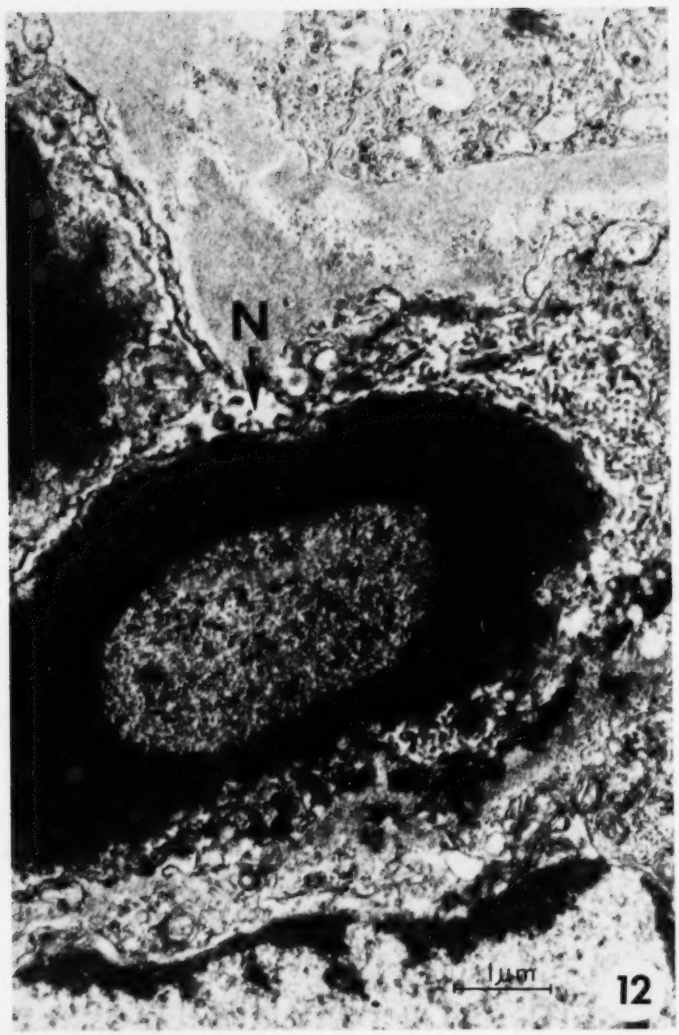

Figure 5-12. Electron micrograph of hemocyte infected with Rhabdo-like virus from the blue crab; note the necrotic nucleus (N) and the displacement of cytoplasmic organelles by the proliferation of virus in the nuclear envelope, and tubular endoplasmic reticulum (micrograph supplied by A. I. Yudin).

More reserach in the area of stress and enhancement of viral infections is needed. Such research may produce insight into virus-host relationships, particularly with regard to using virus-invertebrate systems which are more amenable to experimental manipulation (Couch and Courtney, 1977) than are many vertebrate-virus systems.

REOLIKE VIRUS IN THE BLUE CRAB

Johnson and Bodammer (1975) reported a disease in blue crabs from Chincoteague Bay, Virginia that caused death, and was associated with Reolike virus particles in hemocytes and hemopoietic tissues.

General pathology

Diseased crabs had discolored exoskeltons, and did not feed or molt. Blood from these crabs did not clot well, and paralysis of crabs occurred in a more advanced stage of the disease.

Histopathologic changes found in affected crabs were: necrosis and karyorhexis within hematopoietic tissues; presence of binucleate and multinucleate hemocytes; granular and hyaline hemocytes, and fibroblast like cells within the neural lamellae of the brain of the host; and mild to severe degeneration of nervous tissues (Johnson and Bodammer, 1975).

Ultrastructural changes were somewhat apparent in hematopoietic cells and hemocytes infected with the Reolike virus. Figure 5-13 demonstrates the crystalline arrays of virions in the cytoplasm of a hematopoietic cell. Loss or displacement of normal cytoplasmic organelles are effects of the virus. In some cells almost all cytoplasmic space is filled with either linear arrays of the virus or with randomly distributed virions (Fig. 5-13).

A complex of diseases in crabs is apparently associated with small (ca. 55-60 nm) icosahedral Reolike-viruses and includes the paralysis virus of Macropipus depurator (Bonami, 1973) from Europe, as well as the blue crab virus discovered by Johnson and Bodammer.

PICORNAVIRUS IN PARASITIC ISOPODS AND THEIR CRAB HOSTS

Kuris et al. (1979) described a presumed picornavirus in tissues of a parasitic isopod, Protunion conformis, and its crab host, Hemigrapsus oregonenesis. The hosts were collected from San Francisco Bay, California.

Figure 5-13. Reolike virus in cytoplasm of hemocyte from a blue crab; note loss or displacement of cytoplasmic organelles, and paracrystalline formation of virions within the cytoplasm; the nucleus appears unaffected (micrograph supplied by P.T. Johnson).

General pathology

Cells of the epidermis and hepatopancreas of three specimens of P. conformis had cytoplasmic infection (Fig. 5-14). Two sizes of virus particles were observed in the cytoplasm in crystalline arrays: one was a small particle, 25 nm in diameter, and the other was larger, 58 nm in diameter. The major microscopic and ultrastructural effect of the virus was a complete replacement of normal cellular organelles by the extensive crystalline viral arrays. Nuclei were not affected in infected cells (Fig. 5-14).

Kuris et al. (1979) could not relate virus infection to death in the isopod or crab hosts. They did occasionally find dead isopods on several hosts from populations along the Pacific coast and suggested that studies should be conducted to correlate host mortalities with virus prevalence.

HEXAGONAL VIRUS IN LARVAE OF THE PACIFIC OYSTER

Elston (1979) reported large, hexagonal virus particles (ca. 222 nm) associated with cells in the velar epithelium of hatchery-reared larval Pacific oysters, Crassostrea gigas. The larvae were undergoing serious systemic disease-related changes. Major pathogenic changes in the virus infected cells consisted of: (i) large cytoplasmic vacuoles; (ii) poorly defined cell membranes; and (iii) loss of cytoplasmic homogeneity in the region of virus particles.

More recently, Elston (1980) has related the overall disease syndrome, demonstrated in the larval oysters, to a fungus-like organism that is associated with gastro-intestinal impaction. The virus particles, however, are associated with the above described pathologic changes in the cells in the velar epithelium. Thus, the virus cannot be excluded categorically as a disease agent at the cell level in oysters.

HERPESLIKE VIRUS IN THE AMERICAN OYSTER

Farley et al. (1972) briefly described a herpeslike virus (ca. 70-90 nm) with associated intranuclear inclusions in cells surrounding the blood sinuses in Crassostrea virginica. The oyster hosts were from estuarine waters with elevated temperatures near Wiscasset, Maine, USA. A correlation was found among elevated water temperature (due to effluent from a power plant cooling system), virus infection, and mortality of oysters. Oysters that died in the heated water and which possessed viral inclusion bodies, had the following histopathologic conditions: (i) dilated digestive diverticula; (ii) cellular infiltrates in the vesicular connective tissues about the blood sinuses; and (iii) in

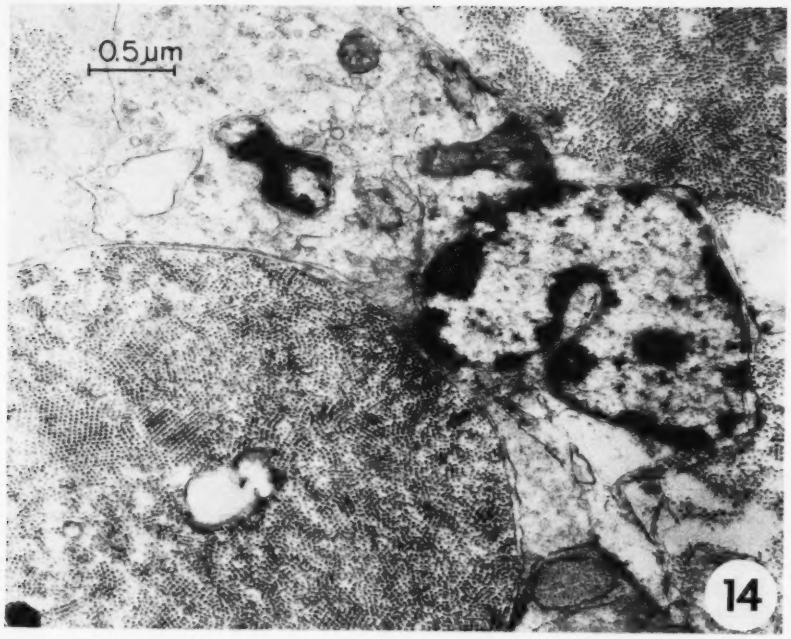

Figure 5-14. Picornavirus filling cytoplasm of epidermis cell from the parasitic isopod, Portunion conformis; note the extensive distribution and abundance of the particles; nucleus of host cell is unaffected. (MIcrograph supplied by A. M. Kuris).

advanced cases, massive cellular aggregates in connective tissues surrounding blood sinuses.

Ultrastructural changes found in the oyster cells consisted of development of virions in the nucleoplasm, presence of large intranuclear inclusions some containing tubules, loss of heterochromatin, and slight nuclear hypertrophy.

The authors suggested that the disease may have a proliferative component, and that elevated water temperature favored the spread or activation, or both, of the virus in oysters. To date, this virus disease has been reported in oysters only from Maine, USA.

PHAGE HYPERPARASITIC IN BIVALVE MOLLUSKS

Buchannan (1978) described a phage-like particle in the cytoplasm of a rickettsia (possibly Coxiella sp.) parasitic in digestive gland cells of the bivalve, Tellina tenuis from various coastal regions of Scotland. Harshbarger et al. (1977) had previously reported phage-like particles from a Chlamydia-like organism from clams and oysters from Chesapeake Bay. This section is concerned with the phage-like particles and related pathology in the rickettsia and its bivalve mollusk host from Scottland.

General pathology

The following descriptive pathologic features reflect both the action of the rickettsia on the bivalve host and the phage on the rickettsia. It is presented this way because future studies may reveal that the pathologic relationship of the rickettsia to the mollusk may be influenced either positively or negatively by the phage (i.e. toxin production mediated by lysogenic phage transfer of a tox gene from rickettsia to rickettsia), even though, at present, there is no evidence for such a role for the phage.

Sections of molluscan digestive gland revealed rickettsia in microcolonies in epithelial cells (Fig. 5-15). Electron microscopy of such microcolonies revealed many rickettsial organisms, most of which contained phage particles (67 nm in diameter) (Fig. 5-17). Some rickettsia were filled with phage (Fig. 5-17, arrow). The major effect of the phage on the rickettsia was to produce pleomorphic forms with low opacity, branching filamentous forms and unusual rod shaped forms of abnormally large size (2-4 μm).

Major pathologic effects of the rickettsia and its contained phage on the host appeared to be hypertrophy of the infected epithelial cell, karyorhexis and karyolysis in infected cells, and compression of the contents of the host cell cytoplasm (Fig. 5-16).

Figure 5-15. Light micrograph of Tellinia tenuis digestive gland tubule from section of epoxy embedded tissue. Note the microcolonies of rickettsia in digestive gland cells (arrows) (micrograph supplied by J. S. Buchannan).

Figure 5-16. Microcolony of rickettsia in the digestive gland epithelium cell of T. tenuis. Note the severe compression of the internal membrane system of the host cell (arrow); note also the presence of phage, visible in this low magnification micrograph, within the rickettsia (micrograph supplied by J. S. Buchanan).

100µm
15
2µm
16

Figure 5-17. Electron micrograph showing rickettsia heavily to lightly infected by phage; note the large (giant) form of rickettsia in the microcolony is heavily infected by phage: also note the rod-like structures in the cytoplasm of the rickettsia associated with the phage (micrograph supplied by J. S. Buchanan).

Buchanan (1978) gave no evidence of lethality of the rickettsia-phage complex in the mollusk.

HEXAGONAL VIRUSLIKE PARTICLES IN TUMORS OF OCTOPUS VULGARIS

Runnger et al. (1971) described large, hexagonal, viruslike particles (ca. 100-400 nm) from edematous, nodular tumors on the arms and bodies of Octopus vulgaris from Naples, Italy. These authors gave a good detailed account of the histopathology associated with the virus and tumors.

General pathology

Edematous tumors from 1 to 10 mm in diameter appeared as nodules on the arms and body in estimated 8.4% of Octopus vulgaris at the Zoological Station in Naples. The tumors were imbedded in muscle tissue (subdermal) and the larger growths may have interfered with normal arm movement.

Histologically, tumors consisted of degenerate muscle fibers surrounded by other myofibers that were in varying stages of degeneration. Muscle cell nuclei in tumors were rounded, enlarged, and sometimes pycnotic. There was an overall picture of concentric necrosis with a fluid filled region at the center of the tumor. No mitotic figures were associated with the tumors. Proximal arteries, connective tissues, and interstitial septa were eventually caught up in the necrotic phenomenon which has its origin in the muscle. Hexagonal, viruslike particles were found between muscle fibers in the tumors. Cellular pathogenic changes associated with the large viruslike particles consisted of multilayered membranes forming around clusters of viruslike particles, and occasional formation of large extracellular inclusion bodies. The viruslike particles themselves resembled the profile of a section through a hexagonal icosahedron. Most particles contained an electron dense core, probably a nucleic acid.

SPHERICAL VIRUSLIKE PARTICLES IN SOMATIC CELLS OF A NEMATODE

Some recent reports have suggested nematodes as possible carriers or vectors of viruses of their hosts, particularly plants (Harrison et al., 1974). Very little has been published on viruses as pathogens of nematodes.

Foor (1972) reported the occurrence of viruslike particles in the somatic cells (hypodermal cells) of Trichosomoides crassicauda, a nematode from the urinary bladder of rats. The viruslike particles were spherical with an electron dense

outer shell and were 15 nm in diameter. The particles were found in dense aggregates in the nucleus of somatic cells.

General pathology

Numerous, amorphous inclusions within the nucleus of hypodermal cells and oviduct cells contained viruslike particles. Cytoplasm of affected cells was vacuolated and contained dense granular elements. Overall appearances of affected cells was that of a degenerate, necrotic cell. The author concluded that there were similarities in degenerate appearance of the nematode cells to degenerate cells in certain insects infected with viruses. No conclusions were presented on the ultimate effects of the viruslike particles on the nematodes.

ADENOVIRUS-LIKE PARTICLES IN EPITHELIAL CELLS OF HYDRA

Viruslike particles that had some characteristics of adenovirus were found in the epithelial cell nuclei to the body of Hydra vulgaris by Bonnefoy et al. (1972). The particles were hexagonal in profile and were 70 to 75 nm in diameter.

General Pathology

Associated with the presence of virus was a general lack of growth and budding (asexual reproduction) in the Hydra. The cessation of growth has been associated with oxygen deficiency and elevated water temperature; therefore, the role of the virus, if any, is uncertain.

Pathogenesis in virus infected cells resulted in nuclear hypertrophy without concomitant changes in nuclear structure. The viruslike particles were found in irregular masses in the nucleoplasm forming plaques of particles that occupy considerable intranuclear space. Bonnefoy et al. (1972) did not speculate on the ultimate effect of the adenovirus-like particles in Hydra.

DISCUSSION

Only thirteen examples of pathologies related to virus or viruslike particles in non-insect invertebrates are described in this chapter. From consideration of these examples, it becomes obvious that detailed descriptions of pathogenesis of virus diseases in non-insect invertebrates has not kept pace

with the frequency of new reports of viruses from these invertebrates (Table 6-1). When possible, it is important for authors reporting viral entities from invertebrates to include as much information as can be collected on the disease syndrome, cellular pathogenesis, physiological and behavioral effects and tissue distribution of the virus. In some cases, information has been so scant that readers are not even certain as to the prevalence of the virus in reported hosts. In some cases only a single report was made with no subsequent papers published. The virus/host examples selected here reflect the rather broad phylogenetic range of non-insect invertebrate hosts now reported to harbor one or more viruses.

Basic information on the nature of non-insect invertebrate virus diseases is needed for several reasons. Foremost is the need to know the range of hosts infected by a particular virus type or group. This not only aids in elucidation of disease etiologies for all animals but also provides clues for potential viral surrogate hosts, reservoirs, or vectors (c.f. papers on phage hyperparasitic in mollusks: Buchanan, (1978); Harshbarger et al. (1977)). Obviously the insights provided into mechanisms of pathogenesis and pathogenic effects in invertebrate viruses may give better understanding of vertebrate virus diseases. Finally, invertebrate virus diseases may be used to investigate fundamental aspects of cellular immunity and disease defense mechanisms in ways that vertebrate-virus systems do not permit.

The development of certain invertebrate virus groups such as the baculoviruses as biological pesticides dictates that the potential host limits of these viruses be determined particularly in non-target invertebrates of close phylogenetic affinity to the natural host. Concurrent with gaining this information will be the opportunity to examine the pathology of such virus groups in newly discovered hosts.

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